PRODUCTION, MODELING, AND EDUCATION

Effects of S6-Strain *Mycoplasma gallisepticum* Inoculation at Ten, Twenty-Two, or Forty-Five Weeks of Age on the Blood Characteristics of Commercial Egg-Laying Hens[1,2]

E. D. Peebles,*[3] E. Y. Basenko,* S. L. Branton,‡ S. K. Whitmarsh,* and P. D. Gerard†

*Department of Poultry Science, and †Experimental Statistics Unit, Mississippi State University, Mississippi State 39762; and ‡Poultry Research Unit, USDA-ARS, Mississippi State, Mississippi 39762

ABSTRACT In 2 consecutive trials of the current study, the effect of the age of application of S6-strain *Mycoplasma gallisepticum* (S6MG) inoculation on the blood characteristics of commercial layers housed and maintained under controlled conditions was determined. The ages of inoculation compared were those before lay at 10 wk of age, during onset of lay at 22 wk of age, and during postpeak lay at 45 wk of age. In each trial, hematocrit, plasma protein, and serum cholesterol, triglycerides, and Ca were determined at 20, 24, 32, 43, 47, and 58 wk of age. The data from both trials were pooled then analyzed together, whereas, data from wk 20 (effect of 10-wk S6MG inoculation); data from wk 24, 32, and 43 (effects of 10- and 22-wk S6MG inoculations); and data from wk 47 and 58 (effects of 10-, 22-, and 45-wk S6MG inoculations) were analyzed separately. At wk 20, hematocrit was higher in birds inoculated with S6MG at 10 wk compared with sham-inoculated birds, and across wk 24, 32, and 43, serum Ca was higher in birds inoculated with S6MG at 10 or 22 wk compared with those that were sham-inoculated. Serum Ca level across wk 47 and 58 was higher in birds inoculated with S6MG at 10 wk compared with sham-inoculated controls and birds inoculated with S6MG at 22 wk, with 45-wk S6MG-inoculated birds being intermediate. The response of serum cholesterol level at 47 wk to S6MG inoculation at either 10, 22, or 45 wk compared with controls was nearly opposite to that of the response observed at 58 wk. However, serum triglycerides were depressed only at wk 47 due to the 45-wk S6MG inoculation compared with all other treatments. Variable postpeak alterations in serum Ca and lipids occur in response to the timing of S6MG inoculation in layers housed under controlled conditions.

Key words: blood, calcium, hematocrit, lipid, *Mycoplasma gallisepticum*

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INTRODUCTION

Mycoplasma gallisepticum (**MG**) infection causes egg production losses in adult laying flocks (Ley, 2003). Live vaccination of commercial layers at 12 wk with F-strain MG (**FMG**) has subsequently been shown to alter liver, ovarian, and uterine characteristics (Burnham et al., 2002b) and yolk total lipid, cholesterol (**CHOL**), and fatty acid concentrations (Burnham et al., 2003b). It has been suggested that alterations in the performance and egg characteristics of laying hens due to FMG (Burnham et al., 2002a) are related to mutual functional disturbances in the liver, ovary, and oviduct (Burnham et al., 2002b) and that FMG colonization in the liver may affect egg production through alterations in yolk total lipid concentration and the metabolism and production of various fatty acids that are ultimately deposited in the yolk (Burnham et al., 2003b).

The various strains of MG may vary in virulence (Garcia et al., 1994), with the S6 strain of MG (**S6MG**) being considered one of the more virulent strains in the field (Levisohn et al., 1986). Deterioration in egg quality has been noted due to infection of the genital tract of chickens inoculated via the yolk sac with S6MG (Pruthi and Kharole, 1981). In companion articles, in which the same housing and population of birds were used as in the current study, S6MG inoculation at 45 wk significantly reduced eggshell quality (Basenko et al., 2005), depressed liver lipid content, and increased relative isthmus weight (Peebles et al., 2006a). In those same birds, S6MG also variably affected postpeak yolk total lipid and fatty acid content in response to the timing of inoculation (Peebles et al., 2006b).

Little is known about events involving factors in the blood that mediate the effects of MG infections on the viability and performance of commercial poultry. In re-

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[3]Corresponding author: dpeebles@poultry.msstate.edu

gards to the interaction of MG and the avian host immune system, Peebles et al. (2004) found no apparent effect of a prelay inoculation of S6MG on the leukocytic profile of commercial layers. However, Ganapathy and Bradbury (2003) showed that a virulent strain of MG caused temporary T-cell suppression in chickens, and FMG infections have previously been shown to cause relative increases in heterophils and monocytes and relative decreases in lymphocyte percentages (Branton et al., 1997). Information concerning the effects of MG infection on the noncellular blood characteristics of birds is lacking in the literature, except for data suggesting that alterations in egg production in response to FMG infection in commercial layers are associated with changes in hematocrit (**HCT**; Burnham et al., 2003a) but are not mediated through changes in circulating very low-density lipoprotein diameters (Burnham et al., 2003c).

The objective of the current study was to compare the effects of S6MG inoculation at 3 different ages throughout a complete egg-laying cycle on the noncellular (exclusive of lipoproteins) blood characteristics of commercial layers. The 3 age periods at which birds were inoculated were before lay (10 wk of age), at onset of lay (22 wk of age), and during lay (45 wk of age). A controlled environment with limited exposure to bacteria and control of temperature, humidity, and lighting (Parker et al., 2002) was used. Isolation units with controlled environments were used to reduce or remove as many environmental stressors as possible so as to more accurately assess the true effect of the organism itself. Comparison of the effects of 10-, 22-, and 45-wk S6MG challenges on various blood factors and their relationships to earlier reported performance traits and reproductive organ and yolk characteristics may provide further information as to the relative effect of S6MG inoculation and its age of application on layer metabolism.

MATERIALS AND METHODS

Pullet Housing and Management

Two individual trials were conducted in this study. In both trials, 1-d-old Single Comb White Leghorn pullets (Hy-Line W-36) were obtained from a commercial source that was monitored and certified free of MG and *Mycoplasma synoviae* (**MS**; National Poultry Improvement Plan and Auxiliary Provisions, 2003). Chicks were vaccinated at 10 d of age for infectious bursal disease via the drinking water. At 12 d, and again at 4 wk of age, chicks were vaccinated for Newcastle disease and infectious bronchitis by the same route. At 9 wk of age in trial 1 and at 6 wk of age in trial 2, 10 randomly selected pullets were bled from the left vena cutanea ulnaris to obtain blood for the serum to be tested for antibodies to MG and MS, using both the serum plate agglutination (**SPA**) and the hemagglutination-inhibition (**HI**) tests (Kleven, 1998). At those same times, swabs were also collected from the choanal cleft (Branton et al., 1984) and placed into tubes containing Frey's-based (Papageorgiou medium) broth (Frey et al., 1968) supplemented with an additional 0.15 mg of Tl acetate and 10^6 IU of penicillin G/mL. Tubes were incubated at 37°C for 30 d or until a phenol red indicator reaction occurred in the media, indicating growth. Media samples from tubes that showed growth were inoculated onto Frey's-based agar and incubated at 37°C. Colonies with morphology suggestive of *Mycoplasma* species were examined by an agar plate fluorescent antibody (**FA**) method (Baas and Jasper, 1972) that used direct labeling of colonies stained with anti-MG polyclonal antibodies produced in rabbits and labeled with fluorescein isothiocyanate (Kleven, 1981).

Until the pullets were 10 wk of age, they were raised on clean, dry litter in a 5.5×6.1 m section of a conventional poultry house at the USDA-ARS Poultry Research Unit, resulting in an initial flock density of 0.034 m^2/bird. A daily artificial lighting schedule followed a cycle of 13L:11D. One 75-W incandescent lightbulb was used to illuminate each 8.4 m^2 of floor space, providing a calculated intensity of 35.5 lx at bird level. At 10 wk of age, 11 pullets were randomly assigned to each of 16 negative-pressure fiberglass biological isolation units (1.16 m^2/unit; total of 176 pullets). The units were housed in a previously described poultry disease isolation facility at the same USDA research laboratory (Branton and Simmons, 1992). All birds were wing-banded for identification and individual data collection. The temperature inside each biological unit was maintained at 25°C.

Layer Housing and Management

Bird numbers in each treatment replicate unit were reduced to 10 per unit (total of 160 pullets) at point-of-lay (18 wk of age) so that bird density was 0.116 m^2/bird for the duration of each trial. Of the 16 total units, 4 replicate units served as sham-inoculated controls throughout the study. Of the 12 other units, 4 replicate units were sequentially assigned to 1 of 3 inoculation treatment groups at the time of inoculation (treatment) so that by wk 45 (last treatment period) of each trial, 4 replicate units were assigned to each of 4 treatments, with each treatment being represented by 1 row of 4 units. More specifically, until the time at which birds in a set of 4 replicate units from a single row were treated (inoculated), they were included as control replicates. Four replicate units received S6MG inoculation at 10 wk of age, and 12 replicate units were designated as sham-inoculated controls from wk 10 through 21. Four of the 12 control units then received S6MG inoculation at 22 wk of age, and 8 replicate units then remained as controls from wk 22 through 44. Lastly, 4 of the 8 control units received S6MG inoculation at 45 wk of age, and 4 replicate units remained as controls from wk 45 through the end of each trial. In trial 2, the location of treatments within the isolation facility was different from that in trial 1 in an effort to remove potential treatment-positioning effects within the facility. Beginning at 18 wk of age, the duration of the artificial lighting schedule was

increased 15 min/d until a cycle of 16 h and 15 min of light per 7 h and 45 min of dark was achieved in trial 1, and a cycle of 17 h and 15 min of light per 6 h and 45 min of dark was achieved in trial 2. These artificial lighting programs were maintained through the end of both trials.

Pullet and Layer Diets

Chickens had ad libitum access to feed and water in each trial. Diets in both trials were formulated according to the age of the birds and included the following: starter (0 to 6 wk), grower (7 to 12 wk), developer (13 to 18 wk), prelay (18 to 19 wk), and layer (20 to 60 wk). All diets were formulated to meet or exceed NRC (1994) recommendations. Ingredient percentages and calculated analyses of these diets were as described by Burnham et al. (2002a). In both trials, CP and Lys percentages in the layer diet were adjusted according to the percentage of feed consumed per bird every 28 d until trial termination. No medication was administered during the study. In a companion article by Basenko et al. (2005), the performance parameters of the flock of birds used in this study were presented. In that article, the determined moisture, ash, CP, crude fat, crude fiber, total N, total digestible N, and fatty acid concentrations of feed and fecal samples collected from these birds at 54 wk of age in trial 2 were reported.

S6MG Inoculation and Mycoplasma Detection

Sham and S6MG inoculation of birds, *Mycoplasma* detection, and titers and passages of the experimental inocula in each trial were as previously described by Basenko et al. (2005). The S6MG strain was tested by a tracheal ring organ culture model, as described by Cherry and Taylor-Robinson (1970) and was shown to significantly reduce ciliary activity within 5 d. To ensure that all treatment groups were equally stressed by the inoculation process and to minimize the possibility of treatment group cross-contamination, control birds were sham-inoculated only once. At 60 wk of age in both trials, 1 randomly selected hen from each replicate unit in each of the 4 treatment groups was bled and swabbed to test for antibodies to and culture for the organism.

Data Collection

In each trial, hens were bled following an overnight fast. Blood was harvested at 20, 24, 32, 43, 47, and 58 wk of age in both trials from 4 hens per isolation unit. Hematocrit, expressed as a percentage of blood packed-cell (primarily red blood cell) volume, was determined by capillary tubes that were centrifuged in a micro-HCT centrifuge and were then read with a microcapillary reader. Serum CHOL and triglycerides (**TRIG**), expressed in milligrams per deciliter, and total plasma protein, expressed in grams per deciliter, were determined by placing 10 μL of serum or plasma for each test on test slides, which were analyzed on a Kodak Ektachem DT-60 analyzer (Eastman Kodak Co., Rochester, NY), as described by Latour et al. (1996). Similarly, serum Ca concentrations, expressed in milligrams per deciliter, were determined by placing 10 μL of serum on a test slide, which was analyzed on a Kodak Ektachem DTSC module analyzer (Eastman Kodak Co.), according to procedures of Tietz (1986). Control analyses were performed to assure that each sample was in the appropriate test range for accurate analysis.

Statistical Analysis

A completely randomized experimental design was utilized. Data at wk 20 (before the 22-wk inoculation; first age interval); from wk 24, 32, and 43 (after the 22-wk and before the 45-wk inoculations; second age interval); and from wk 47 and 58 (after the 45-wk inoculation; third interval) were analyzed separately. The data of both trials were pooled and then analyzed together. Trial was taken to be a random effect. Data within the first age period were subjected to 1-way analysis, because only 1 age period was considered. All data within the last 2 age intervals were subjected to a repeated measures analysis to account for the fact that the same experimental units were observed over multiple age periods. In the first age interval, control and 10-wk S6MG-inoculated groups were compared. In the second age interval, control, 10-wk S6MG-inoculated, and 22-wk S6MG-inoculated groups were compared. In the third age period, control, 10-wk S6MG-inoculated, 22-wk S6MG-inoculated, and 45-wk S6MG-inoculated groups were compared. Individual sample data within each replicate unit were averaged before analysis. Results from trials 1 and 2 were not reported independently but were reported over both trials. Least squares means were compared in the event of significant global effects (Steel and Torrie, 1980). All data were analyzed using the MIXED procedure of SAS software (SAS Institute, 2000). Statements of significance were based on $P \leq 0.05$, unless otherwise stated.

RESULTS

All initial mycoplasmal cultures, as well as SPA and HI test results obtained from pullets at 9 wk of age in trial 1 and at 6 wk of age in trial 2, were negative for MG and MS. Serum samples obtained from control birds at 60 wk of age in both trials were SPA- and HI-negative for MG, whereas the same tests were positive for MG in the S6MG-inoculated hens. Hens were considered MG-free when they exhibited no detectable HI titers. All S6MG-inoculated hens tested had HI titers ≥ 1:80 (geometric mean of 80.0). Similarly, FA culture results for swabs obtained at 60 wk of age in both trials were negative for *Mycoplasma* species growth for all control hens tested, whereas all S6MG-inoculated hens tested

Table 1. Hematocrit (HCT) at 20 wk (first age interval; HCT-1); serum Ca (Ca-2) across 24, 32, and 43 wk of age (second age interval); and serum Ca (Ca-3) across 47 and 58 wk of age (third age interval) in sham-inoculated control (control) and in 10-, 22-, and 45-wk S6-strain *Mycoplasma gallisepticum* (S6MG-10, S6MG-22, S6MG-45) inoculation treatments, trials 1 and 2[1]

Treatment	HCT-1 (%)	Ca-2	Ca-3
		(mg/dL)	
Control	28.6 ± 0.44[b,1]	27.2 ± 3.04b[b,2]	28.2 ± 1.64[b,3]
S6MG-10	29.6 ± 0.54[a,3]	30.1 ± 3.11[a,3]	32.7 ± 1.67[a,3]
S6MG-22	—	29.4 ± 3.11[a,3]	28.4 ± 1.64[b,3]
S6MG-45	—	—	31.7 ± 1.64[ab,3]

[a,b]Means among treatments within column with no common superscript differ significantly ($P \leq 0.05$).

[1]n = 12 replicate units used for calculation of mean.

[2]n = 8 replicate units used for calculation of mean.

[3]n = 4 replicate units used for calculation of mean.

Table 2. Serum cholesterol-3 (CHOL-3) and triglycerides-3 (TRIG-3) in sham-inoculated control (control) and in 10-, 22-, and 45-wk S6-strain *Mycoplasma gallisepticum* (S6MG-10, S6MG-22, S6MG-45) inoculation treatments across 47 and 58 wk of age (third age interval), trials 1 and 2[1]

	Week			
	CHOL-3[2]		TRIG-3[3]	
Treatment	47	58	47	58
	(mg/dL)			
Control	199[a]	162[b]	3,728[a]	3,194
S6MG-10	169[ab]	195[a]	3,371[a]	3,343
S6MG-22	200[a]	151[b]	3,921[a]	3,039
S6MG-45	157[b]	164[ab]	2,555[b]	3,338

[a,b]Means among treatments within column with no common superscript differ significantly ($P \leq 0.05$).

[1]n = 4 replicate units used for calculation of mean for each parameter within each treatment.

[2]SEM based on pooled estimate of variance = 21.1.

[3]SEM based on pooled estimate of variance = 282.4.

were MG-positive (positive for MG fluorescence) and MS-negative (negative for MS fluorescence).

In the first age interval (wk 20, or before the 22-wk inoculation), there was no significant effect due to the 10-wk S6MG inoculation on any of the blood parameters examined, except for HCT (only HCT data presented). At wk 20, HCT was higher ($P \leq 0.05$) in birds inoculated with S6MG at 10 wk compared with sham-inoculated birds (Table 1). In the second age interval (after the 22-wk and before the 45-wk inoculations), there were no significant main effects or interactions due to bird age or S6MG inoculation treatment (10 or 22 wk) on HCT, CHOL, TRIG, or total plasma protein (data not presented). However, serum Ca was higher ($P \leq 0.02$) in birds inoculated with S6MG at 10 or 22 wk compared with those that were sham-inoculated (Table 1).

In the third age interval (after the 45-wk inoculation), there were no significant bird age main effects on any of the parameters investigated (data not presented). Furthermore, there were no significant inoculation treatment main effects or bird age by treatment interactions on HCT or total plasma protein (data not presented). However, there was an inoculation treatment main effect ($P \leq 0.05$) on serum Ca (Table 1), and there were bird age by treatment interactions for CHOL ($P \leq 0.01$) and for TRIG ($P \leq 0.05$; Table 2). Serum Ca level in the third age interval was higher in birds inoculated with S6MG at 10 wk compared with sham-inoculated controls and birds inoculated with S6MG at 22 wk, with 45-wk S6MG-inoculated birds being intermediate (Table 1). At 47 wk, CHOL was lower in birds inoculated with S6MG at 45 wk compared with sham-inoculated and 22-wk S6MG-inoculated birds, with intermediate levels in birds inoculated with S6MG at 10 wk (Table 2). Conversely, at 58 wk of age, CHOL was higher in birds inoculated with S6MG at 10 wk compared with sham-inoculated and 22-wk S6MG-inoculated birds, with intermediate levels in birds inoculated with S6MG at 45 wk (Table 2). At 47 wk, TRIG was lower in birds inoculated with S6MG at 45 wk compared with that in birds from the other 3 treatment groups (Table 2). For sake of conciseness, only those data for which a significant effect was noted are presented.

DISCUSSION

At the end of this study (60 wk of age in both trials), SPA, HI, and FA tests verified systemic infections in S6MG-inoculated birds. Conversely, sham-inoculated birds remained S6MG-free throughout the study. Manifestations of MG usually occur in the respiratory system, and lesions become extensive when complicated by other bacteria. Furthermore, environmental factors such as dust and ammonia, along with intensive rearing or stress, crowding, cold weather, live-virus vaccination, or natural virus infection may also be important in lesion incidence and severity (Jordan, 1972; Springer et al., 1974; Jordan, 1985). However, when there are no secondary infections, mycoplasmosis is often subclinical or mild (Kerr and Olson, 1967). The birds in this study were housed in biological isolation units from 10 wk of age through the remainder of the study, where they remained discernibly free of natural infections and other environmental stressors common in commercial operations. As a result, the S6MG-infected hens exhibited no outward pathological symptoms. Furthermore, as reported by Basenko et al. (2005), S6MG inoculation treatment had no significant effect on either the mortality or BW of these same hens.

Parker et al. (2002) examined the effects of a 10-wk S6MG inoculation on the performance and egg characteristics of hens housed similarly to those in this study. It was reported that egg production, egg weight, eggshell quality, and the percentage of yolk weights of eggs from 24 to 50 wk were not affected by the 10-wk S6MG inoculation. In a companion article by Peebles et al. (2004), it was reported that the 10-wk S6MG inoculation also had no apparent effect on leukocyte profile. No other properties of the blood were examined in those birds; however, the current results do show that a 10-wk S6MG inoculation may cause a deferred but continuous elevation in

serum Ca levels during lay from 24 to 58 wk of age. Parker et al. (2003) noted in a different study using similarly housed hens that S6MG inoculation at 20 wk also had no affect on egg production from 22 to 55 wk or on the relative weights of the liver and ovary at 57 wk. Nevertheless, S6MG inoculation at 20 wk did significantly affect the lengths and weights of various portions of the reproductive tract. More specifically, significant decreases were noted in the lengths of the oviduct, infundibulum, and magnum, whereas uterus weight was lower and vagina length was increased in S6MG-inoculated hens. The blood characteristics of the birds that were subjected to the 20-wk S6MG inoculation in the report by Parker et al. (2003) were not examined. However, effects on the reproductive tract after the 20-wk inoculation would suggest that there may be associated effects on the blood characteristics of those birds.

Results from trials in which the effects of a 12-wk FMG inoculation on the blood characteristics of laying hens were examined (Burnham et al., 2003a) suggested that alterations in egg production in response to FMG infection, as noted in a previous report (Burnham et al., 2002a), may be associated with changes in HCT. In both trials conducted by Burnham et al. (2003a), HCT at 20 wk of age was significantly increased in birds inoculated with FMG at 12 wk. Similarly, in the current study, the 10-wk S6MG inoculation significantly elevated HCT at 20 wk. By 8 to 10 wk after challenge (20 wk), inoculation with either FMG or S6MG may confer a compensatory polycythemic response to the insult imposed on the respiratory system by either strain of MG. Conversely, after 20 wk, HCT levels returned to those similar to controls, which would indicate that the hens were able to eventually adjust through other physiological means to the MG challenge (Burnham et al., 2003a). Nevertheless, this result confirms that the initial weeks of egg production are stressful to layers, particularly when combined with the onset of a full systemic MG infection.

The effects of a 12-wk FMG inoculation on reproductive organ (Burnham et al., 2002b), egg yolk (Burnham et al., 2003b), and serum lipoprotein (Burnham et al., 2003c) characteristics of the same birds used by Burnham et al. (2002a) were determined. It was suggested by Burnham et al. (2003a) that, despite the lack of change in circulating lipoprotein characteristics, postpeak decreases in TRIG and plasma protein may have been associated with ovarian follicular regression, reproductive tissue atrophy, and the onset of fatty liver hemorrhagic syndrome. It was further suggested that decreased concentrations of lipids in the blood may be directly responsible for reductions in yolk total lipid, CHOL, and fatty acid deposition. Delayed onset of lay and decreases in weekly egg production after 42 wk and average weekly egg production throughout lay after a 12-wk FMG infection, as noted by Burnham et al. (2002a), apparently caused mutual functional disturbances in reproductive tract function and the respective metabolism and deposition of circulating lipids in the liver and ovarian follicles.

In a companion article, in which the same birds were used as in this study, Basenko et al. (2005) reported that 10-, 22-, or 45-wk S6MG inoculations had no effect on egg production, egg weight, or relative yolk and albumen weight from 24 to 58 wk. However, across 47 and 58 wk, both eggshell weight per unit of surface area and percentage of eggshell weight were significantly lower in eggs laid by hens inoculated with S6MG at 45 wk compared with eggs laid by hens that had not received an S6MG inoculation or that were inoculated with S6MG at 10 or 22 wk of age. Because only the 10-wk inoculation led to a significant change in serum Ca levels from that of controls across wk 47 and 58 in the current study, it would appear that the effects of the 45-wk inoculation on subsequent eggshell quality during that same period were not related to serum Ca. Nevertheless, in these same birds, Peebles et al. (2006a) reported that liver lipid concentration was depressed, and isthmus weight, as a percentage of total oviduct weight, was increased at 60 wk in response to only the 45-wk S6MG inoculation. Basenko et al. (2005) suggested that layers may be better able to adapt to an S6MG infection when inoculated before or at the onset of lay rather than late in lay. The decrease in liver lipid content in response to the 45-wk inoculation compared with the absence of an effect after the 10- or 22-wk inoculations reported by Peebles et al. (2006a) would further indicate that livers of commercial laying hens are more susceptible to the effects of S6MG infection at 45 wk than at prelay or at the initiation of lay. An active S6MG infection may, therefore, cause livers in birds to react earlier in the reproductive cycle in ways similar to that of inefficient or aging hens.

Peebles et al. (2006b) found, using the same birds as those used by Basenko et al. (2005) and Peebles et al. (2006a), that, despite the singular effect of the 45-wk inoculation on liver lipid content, the 10-wk prelay inoculation depressed yolk total lipids compared with the inoculation given late in lay (45 wk), and both the early (22 wk) and late (45 wk) inoculations during lay changed the concentrations of various yolk fatty acids in relation to concentrations after the prelay inoculation. Therefore, the effects of S6MG inoculation timing on liver lipid concentration do not directly correspond to those effects on yolk lipid content. On the other hand, the 45-wk inoculation did depress yolk myristic, oleic, linolenic, and linoleic acid concentrations but increased yolk stearic and arachidonic levels compared with controls. Alterations in liver lipid metabolism, the transport and delivery of lipids to the ovary, or the deposition of lipid in developing ovarian follicles are possible means by which differences in yolk lipid may occur. Although it was concluded in the report by Peebles et al. (2006a) that the incidence of fatty liver hemorrhagic syndrome was not influenced by S6MG inoculation, the observed effects on liver and yolk lipids would target liver lipid metabolism and lipid uptake by ovarian follicles as probable mechanisms through which subsequent blood lipid content may be affected by an S6MG infection. Concomitant decreases in liver total lipid and yolk fatty acid contents

would, more specifically, suggest that decreases in CHOL and TRIG in the current report would be due to a disruption of lipid metabolism in the liver. Recent findings show that MG has the ability to invade cells (Winner et al., 2000), suggesting that MG may be capable of interfering with liver lipid metabolism. Furthermore, Winner et al. (2000) demonstrated that MG can pass through the mucosal barrier to cause systemic infections, and MG may be cultured from the avian liver (Sahu and Olson, 1976).

In conclusion, results from the current study and that conducted by Burnham et al. (2003a) indicate that prelay inoculations of MG (i.e., FMG at 12 wk of age or S6MG at 10 wk of age) result in an increase in HCT in laying hens early in lay (20 wk of age). The current results alone further suggest that an S6MG inoculation at 10 wk of age may result in an elevation in serum Ca levels during lay and that an S6MG inoculation at 45 wk of age may cause proximate (by 47 wk of age) decreases in CHOL and TRIG via a disruption of liver lipid metabolism.

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